Info Sheet: **Glycan/Glycoprotein analysis**

Identification of glycoproteins

General description:

The N-glycosylation occurs at asparagine residues in the consensus sequence N-X-S/T (X≠P).
The workflow consists of:

- Glycoprotein enrichment with specific protocols
- Protein digestion (Trypsin) to generate (glyco)peptides
- LC-MS analysis
- Data analysis
    - Protein identification (Mascot) and visualization of protein identities (Scaffold)
    - Filtering for proteins with consensus sequence N-X-S/T (X≠P).
- Expected turnaround time (after arrival of samples):
    - 3-7 days

Requirements and considerations:

- The enrichment of glycoproteins should be optimized and performed by the customer.
- submit samples as Coomassie Blue stained gel bands (whenever possible avoid silver staining) or in solution (free of protease inhibitors!)

N-linked glycans site occupancy

General description:

A glycoprotein may contain a number of potentially N-glycosylated sites, each of which may or may not be glycosylated.
The workflow consists of:

- Glycoprotein enrichment with specific protocols
- N-Glycan release using PNGase F or A (OPTION: in $H_2{}^{18}O$ water)
- Protein digestion (Trypsin) to generate peptides
- LC-MS analysis
- Data analysis
    - Protein identification (Mascot) and visualization of protein identities (Scaffold)
    - Filtering for consensus sequence N-X-S/T (X≠P) with deamidated N.
- Expected turnaround time (after arrival of samples):
    - 5-10 days

Requirements and considerations:

- The enrichment of glycoproteins should be optimized and performed by the customer.
- The usage of $H_2{}^{18}O$ water significantly reduces the number of false positive results.
- For the usage of $H_2{}^{18}O$, please contact us at proteomics@fgcz.ethz.ch

Site-specific glycosylation

General description:

The N-Glycan microheterogeneity can be studied analysing what kind of glycan is attached to which site of a protein.
The workflow consists of:

- Glycoprotein enrichment with specific protocols
- Protein digestion to generate (glyco)peptides
- LC-MS analysis using different fragmentation techniques
- Data analysis
    - Glycopeptide identification using Mascot or Byonic
    - Manual validation by visual inspection of spectra
- Expected turnaround time (after arrival of samples):
    - 5-15 days

Requirements and considerations:

- The enrichment of glycoprotein should be optimized and performed by the customer.
- The manual inspection is required, depending on availability of a glycan database for a given species.

Characterization of N- and O-linked glycans

General description:
A glycome often comprises a repertoire of closely related structures, many of which are structural isomers. The scope is to know detailed glycan structures or general glycan profiling.
The workflow consists of:

- Glycoprotein enrichment with specific protocols
- N-Glycans: release using PNGase F or A / O-Glycans: β-elimination/reduction reaction
- Chemical derivation, mainly using the permethylation reaction
- MALDI-MS analysis
- Data analysis
    - Glycan identification using ProteinScape 3.0 and Glycoworkbench 2
    - Annotation of the glycan structures and their absolute intensities
- Expected turnaround time (after arrival of samples):
    - 10-15 days

Requirements and considerations:

- The enrichment of glycoprotein should be optimized and performed by the customer.
- The analysis of N-Glycans can be offered as a service, while the analysis of O-Glycans is currently handled only via User Lab.